Application of Direct Current to Protect Bioreactor against Contamination

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Bacteria cell suspensions were sterilized by direct electric current and the cell death rate was proportional to the current. When repeated hydrolysis of casein by immobilized mycelia was done under 0.06 A of direct electric current, contaminants were inhibited while the hydrolysis was stable for more than 10 batches (250 h).

Sterilization is very important during bioprocesses. Many methods such as heat sterilization, filter sterilization, and addition of chemicals are currently used to prevent the growth of contaminants. However, it is still necessary to develop a more effective and simple method of sterilization.

Recently, the effects of direct current on the growth of bacteria have been investigated. It has been postulated that bacterial cells are killed by toxic substances such as free chlorine, H₂O₂, and pyrophosphate, which are generated by the electrode reaction. Another view is that the cells are killed physically by the impulse wave of high voltage. Although some reactors for sterilization by electric current have been proposed, there are yet no reports on methods of preventing contamination using direct currents that are directly incorporated into a bioreactor.

In this paper, the effects of direct current on the sterilization and growth inhibition of the bacteria were investigated. In addition, the stability of the bioreactor under applied direct current was examined by repeated casein hydrolysis with immobilized mycelia.

Various kinds of microorganisms, including Gram-positive, Gram-negative, and food-poisoning bacteria, were used for investigations on the effects of direct current on microbial cells. Bacillus subtilis IFO 3309, Escherichia coli IFO 3301, Staphylococcus aureus ATCC 6538P, and Pseudomonas aeruginosa IFO 12689 which were obtained from Culture Collection Center, Tokyo Univ. of Agriculture, as well as contaminants originating from added casein solution were tested. Each bacterial strain was cultivated in shake flasks (125 oscillations/min) containing 100 ml of nutrient broth medium (pH 7.0) at 30 C for about 15 h.

In the sterilization experiments, 5 ml of the bacterial culture broth were mixed with 225 ml of N/30 phosphate buffer (pH 7.0) resulting in an initial cell concentration of 10⁸ cells/ml. In the study on the effects of direct current on the growth of contaminants, 0.5% (w/v) casein solution containing 10⁴ cells/ml was used. The cell suspension was inoculated into the reactor at 30 C and direct current was applied from the power supply (Fig. 1). The number of living cells in the solution was measured every two hours by plating appropriately diluted solution on 0.8% (w/v) agar-nutrient medium. The colonies were counted after two days of incubation at 30 C.

Immobilized Asp. oryzae AHU7141 was prepared by the method described previously. Repeated hydrolysis of casein by immobilized mycelia was done in a stirred 0.5 liter-reactor (Fig. 1). The working volume and pH were 0.3 liter and 7, respectively, while the initial concentration of casein was 0.5%. The casein solution was sterilized by autoclaving at 121 C for 10 min. The casein hydrolysis was repeated by replacing the reaction mixture with a fresh one when the amino acid concentration increased to about 300 mg/liter. The amino acid concentration was measured according to the Folin method. Heat sterilization of the reactor was done only for the first batch reaction.

When direct current was applied, the surviving fraction (living cell concentration/initial cell concentration) decreased with the lapse of time, but it remained constant in the control (without

Fig. 1. Schematic Diagram of the Electrically Controlled Bioreactor. 1. power supply, 2. magnetic stirrer, 3. Pt-electrode. The area of each Pt-electrode is 0.8 cm² and the distance between them is 5.5 cm.

Fig. 2. Survival Curves of Bacteria. (a) E. coli; (b) B. subtilis; (c) Pseudomonas aeruginosa; (d) Staphylococcus aureus. Symbols: ○, control; ▲, DC 5 V(0.02 A); ◀, DC 15 V(0.15 A); ■, DC 30 V(0.33 A).

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applied direct current). When 0.33 A of direct current was applied, the surviving fraction of *E. coli* decreased to 1% of the initial value after 1 h (Fig. 2(a)). Direct current was also effective for sterilization of *B. subtilis*, *P. aeruginosa*, and *S. aureus* cells as shown in Figs. 2(b)–(d). In all these cases, the rate of decrease in the surviving fraction increased as the electric current increased. However, the time required for sterilization varied with the cells and this may be due to differences in the cell structure. The results for contaminants which may be many types of bacteria are summarized in Fig. 3. The result shows that the growth of contaminants was equally inhibited by direct electric current. In this system, the effects of electric current on the cells seem to be similar to those already reported.3–10)

Since there were no nutrients in the reaction mixture in these systems, bacterial growth was not observed even when there was no direct current. This is, however, different from the normal bioreactor systems where media or reaction mixtures rich in nutrients are used. In such cases, once a bioreactor is contaminated, rapid growth of the contaminants takes place. It is, therefore, necessary not only to initially sterilize the media and the reactor but also to inhibit the growth of cells which may accidentally contaminate the reactor during the bioprocess.

As shown in Fig. 4, the length of the lag phase increased with increase in the electric current and growth was completely inhibited by 0.06 A of direct current.

These results have shown that by applying direct current to bacterial suspensions, it is possible to sterilize it or to inhibit the growth of bacteria. The possibility of protecting a bioreactor from contamination by applying direct current to the reaction mixture was therefore investigated.

When repeated hydrolysis of casein was done under ordinary conditions (no direct current), contamination occurred and the concentration of contaminants increased to about $10^6$ cells/ml during the third batch reaction. Furthermore, the activity of the immobilized mycelia decreased following the growth of the contaminants (Fig. 5). On the other hand, when 0.06A of direct current was applied to the reaction mixture, contaminants were not detected and the hydrolysis reaction was stable for more than 10 batch reactions (Fig. 6). Furthermore, direct current did not influence the casein hydrolysis activity of immobilized mycelia during the operation periods.

We consider that this method of using direct current for protection of bioreactor from contamination can also be applied to other bioprocesses.

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References


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